as a single amendment paper, which is attached to this response. Also attached is the compare copy of the claims, marked to show all of the changes relative to the previous version of the claims.

REMARKS

Claims 1 to 30 are pending. Claims 19 and 24 have been amended to specify that the fibroblast cells are stimulated "by culturing the cells" in a second culture medium. Support for these amendments is found in original claim 27. No new matter has been added by these amendments which are made to better define the claimed invention and entry of these amendments is respectfully requested.

Applicants acknowledge that the rejections under 35 U.S.C. § 112, first paragraph have been withdrawn.

Applicants assume that any rejections not repeated in the outstanding office action have been withdrawn.

The remaining rejections are addressed below.

I. Rejections Under Section 112, Second Paragraph.

Claims 1-30 are rejected for various reasons as being unclear under § 112, second paragraph. Applicants respectfully traverse these rejections. The individual rejections are discussed below.

A. Claims 1-30 are rejected under § 112, second paragraph, as being unclear what is the nature of "exogenous matrix components" and what is encompassed by "synthetic members."

This invention is directed to an *in vitro* cultured tissue construct of cultured cells and endogenously produced extracellular matrix components. The invention is also directed to the method for producing this cultured tissue construct.

Prior art tissue constructs were constructed with a variety of techniques, but all of these prior art techniques employed either exogenous matrix components or synthetic members during the culturing conditions, or both. (See specification, page 3, lines 5-7, "Heretofore, current

engineered living tissue constructs are not completely cell assembled and must rely on either the addition or incorporation of exogenous matrix components or synthetic members for structure or support, or both.")

For instance, one of the cited prior art references, Bell, U.S. 4,485,096, teaches how to make a skin-equivalent by growing keratinocytes on contracted collagen lattices. Bell teaches that keratinocytes can be plated at the time the matrix gel forms, at any time while the lattice is contracting, or at any time after the contraction is completed (col. 4, lines 19-22). The keratinocytes form a confluent layer on the lattice surface (col. 4, line 24). This teaching shows the prior art reliance on exogenous matrix components, such as the contracted collagen lattices described in Bell. In the instant application the extracellular matrix is produced by the cultured cells in the absence of exogenous matrix components or synthetic members.

The specification of the instant application clearly states that exogenous matrix components are matrix components not produced by the cultured cells but introduced by other means (page 4, lines 6-7). Thus, the matrix is "completely cell-synthesized and assembled by culturing the cells" (page 4, lines 3-4).

The specification further states that synthetic members are used for structure or support or both. (page 3, line 7). An example is a mesh member for the formation of the tissue constructs (page 10, lines 21-29).

Applicants submit that the terms "exogenous matrix components" and "synthetic members" are clear in light of this description in the specification. For these reasons, the Examiner is requested to reconsider and withdraw the rejection.

B. Claims 7 and 21 are rejected under § 112, second paragraph, as being unclear as to the metes and bounds of "no non-human components."

The specification at page 2, lines 1-2 elucidates the phrase "no non-human components" by stating that the methods of the invention can be carried out "without the use of undefined or non-human-derived biological components, such as bovine serum or organ extracts." In addition, the specification at page 12, lines 1-8, says:

> When the invention is carried out utilizing screened human cells cultured using chemically defined components derived from no non-human animal sources, the resultant tissue construct is a defined human tissue construct. Synthetic functional equivalents

may also be added to supplement chemically defined media within the purview of the definition of chemically defined for use in the most preferred fabrication method. Generally, one of skill in the art of cell culture will be able to determine suitable natural human, human recombinant, or synthetic equivalents to commonly known animal components to supplement the culture media of the invention without undue investigation or experimentation.

For these reasons, the Examiner is requested to reconsider and withdraw the rejection.

C. Claims 19-23 and 24-26 are rejected under § 112, second paragraph, as being unclear as to the steps involved in "stimulating" the fibroblast cells to synthesize, secrete, and organize extracellular matrix components.

Claims 19 and 24 have been amended to specify that the fibroblast cells are stimulated "by culturing the cells" in a second culture medium. This language is included to better define the claimed invention and is not considered to narrow the amended claims.

For these reasons, Applicants submit that this rejection has been overcome, and the Examiner is requested to reconsider and withdraw the rejection.

D. Claims 1-18, 28, and 30 are rejected under § 112, second paragraph, as being unclear as to the metes and bounds of cultured "under conditions to produce a layer of extracellular matrix."

It is well-established that the determination whether a claim is invalid as indefinite depends on whether those skilled in the art would understand the scope of the claim when the claim is read in light of the specification. The specification clearly describes the supplementation of the medium with components that assist in matrix synthesis, secretion, or organization (page 16, line 3 to page 17, line 24). The specification additionally describes the environmental conditions of controlled temperature, humidity, and gas mixture in which the cultures are maintained (page 17, lines 25-28). Therefore, one skilled in the art would understand the scope of this claim language when read in light of the specification.

For this reason, the Examiner is requested to reconsider and to withdraw this rejection.

II. Rejections Under Section 103.

A. Claims 1-3, 6-12, and 19-27 are rejected under Section 103 as unpatentable over Bell, U.S. 4,485,096, Parenteau et al., U.S. 5,712,163, Sand, U.S. 5,618,284, Holbrook et al. (1993) and Biegel et al. (1994).

Applicants note that this rejection does not use the language "in view of," which would indicate a combination rejection. For this reason Applicants previously argued against the references separately. Although the same language is used for the rejection in the outstanding Office Action, based on the discussion of the references in the outstanding Office Action, it appears as if the references are being treated as secondary references. Therefore, Applicants will herein address this rejection as a rejection over the cited reference Bell in view of the cited references Parenteau, Sand, Holbrook, and Biegel.

Applicants respectfully traverse this rejection. Applicants are well acquainted with the cited references Bell and Parenteau as these patents are licensed and assigned, respectively, to the assignee of the instant application.

Applicants note that the Office Action mentions the use of a Transwell plate coated with collagen on pages 5 and 6. As discussed in the Office Action filed April 12, 2001, Transwell plates are available with and without a collagen coating. The Examiner has mentioned a collagen coated product line, not the polycarbonate or polyester lines that are not coated. The specification indicates at page 10, lines 7-9 that for human dermal fibroblasts, the most preferred material is polycarbonate. Furthermore, use of a Transwell plate, much less one coated with collagen, is not required by the claims.

The instant invention generally claims a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises specified components and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions as well as methods for producing this cultured tissue construct.

The patentable distinction between the Bell patent and the instant application is that Bell requires the use of a hydrated lattice that contains an exogenous matrix component. As stated in

the Office Action, "Bell et al does not teach the use of chemically defined media, the molecular composition of the differentiated tissue, e.g. collagen, decorin, and GAG, or the use of said procedure in the absence of exogenous matrix components (e.g. collagen)" (Office Action, page 4).

Parenteau et al. does teach a chemically defined cell culture medium, but does not teach or suggest the instantly claimed invention. The Office Action points to Example 5 of Parenteau et al. as demonstrating that Parenteau et al. teaches the culturing of human keratinocytes on plates not coated with collagen. Example 5 of Parenteau et al. relates to a study to determine actual substrate dependence and the effect of substrate over culture lifespan. The results of this study indicate that cells plated on dishes with collagen generally performed better than those plated on dishes without collagen. The study found that: (1) cells established from frozen primary cultures without collagen showed a slight decrease in plating efficiency over those plated on collagen; (2) as the cells without collagen were further passaged onto dishes with and without collagen, the plating efficiency was consistently better on those plated onto dishes with collagen; (3) cells initially maintained with collagen and subsequently passed onto dishes with or without collagen showed variable plating efficiencies without clear advantage or disadvantage to the use of collagen; and (4) cells grown and passed onto collagen took longer to show a drop in plating efficiency than those grown without collagen.

An additional experiment discussed in Example 5 relates to a strain of epidermal cells in high serum with 3T3 feeder cells. As discussed earlier in the specification of Parenteau et al. at column 2, lines 38-58, this method uses a 3T3 feeder cell layer (from a transformed mouse cell line) to allow clonal growth and multiple passage and therefore does not teach the claimed invention.

Therefore, Example 5 of Parenteau et al. teaches that cells plated on dishes with collagen performed better than those plated on dishes without collagen. Furthermore, Parenteau et al. states that "[v]arious substrates can be used in the practice of the present invention . . . . In the claimed systems, growth may be enhanced under certain conditions using either fibronectin or collagen coated substrate . . . . The presence of a substrate, e.g., a matrix component appears to allow the cells to establish colonies when conditions are less than optimal or more stringent . . . ." (col. 18, line 63 to col. 19, line 16). Thus, one would not be motivated to combine the Parenteau et al. reference with Bell in order to culture cells without collagen because Parenteau

et al. would suggest the use of collagen, not its absence, as being advantageous. Parenteau et al. do not teach that the cells cultured in Example 5 produce their own matrix. Parenteau et al. do not show a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells or an extracellular matrix produced by the cultured fibroblast cells.

Parenteau et al. generally teaches the production of a culture of epidermal cells. In Parenteau et al. a living skin equivalent was fabricated by plating keratinocytes onto a dermal equivalent made according to established procedures (col. 26, lines 1-4), thus using exogenous matrix components. The tissue cultures in the instant invention produce their own extracellular matrix and form a tissue construct. The ability of fibroblast cells to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members was unexpected. The cells cultured in Example 5 of Parenteau et al. would produce merely a multitude of epidermal cells, not a tissue construct. Therefore, Parenteau et al. do not teach a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells wherein the extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members.

Sand states what is known in the art, that is, that human type-1 collagen molecule consists of chains of 300 nm triple helixes joined by 67 nm uncoiled bonds. Sand does not teach a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells wherein the extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members.

Holbrook et al. also state what is known in the art, that is that the dermal matrix of connective tissue is comprised of collagen (of which 80-90% is type I and 8-12% is type III) glycosaminoglycan, fibronectin, and tenascin. Holbrook et al. does not teach a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells wherein the extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members.

Biegel et al. does describe coating transwell filters with hydrated collagen gels and then culturing endothelial cells. The hydrated collagen gel is similar in principle to the hydrated lattice of Bell, above, and is an exogenous matrix component. Therefore, Biegel et al. does not teach a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells wherein the extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members.

As stated by the Federal Circuit in *Smiths Industries Medical Systems, Inc. v. Vital Signs, Inc.*, 183 F.3d 1347 (Fed. Cir. 1999):

> [T]here is no basis for concluding that an invention would have been obvious solely because it is a combination of elements that were known in the art at the time of the invention. See Fromson v. Advance Offset Plate, Inc., 755 F.2d 1549, 1556, 225 USPQ 26, 31 (Fed. Cir. 1985). Instead, the relevant inquiry is whether there is a reason, suggestion, or motivation in the prior art that would lead one of ordinary skill in the art to combine the references, and that would also suggest a reasonable likelihood of success. See, e.g., In re Dow Chem. Co., 837 F.2d 469, 473, 5 USPQ2d 1529,1531-32 (Fed. Cir. 1988). Such a suggestion or motivation may come from the references themselves, from knowledge by those skilled in the art that certain references are of special interest in a field, or even from the nature of the problem to be solved. See In re Rouffet, 149 F.3d 1350, 1355-56, 47 USPQ2d 1453, 1456 (Fed. Cir. 1998); Pro-Mold & Tool Co. v. Great Lakes Plastics, Inc., 75 F.3d 1568, 1573, 37 USPQ2d 1626, 1630 (Fed. Cir. 1996).

Id. at 1356.

Therefore, it is submitted that one skilled in the art would *not* be motivated to combine the cited references to produce the claimed invention. The instantly claimed invention is directed to bioengineered tissue constructs of cultured cells and endogenously produced extracellular matrix components without the requirement of exogenous matrix components or synthetic members. Therefore, Applicants submit that the cited references do not, either alone or in combination, render the claimed invention obvious. For this reason, the Examiner is requested to reconsider and to withdraw this rejection.

B. Claims 1, 4, 5, 9, 13, and 14 are rejected under Section 103(a) as obvious over Jahoda et al. (1993) in view of Parenteau et al., U.S. 5,712,163.

Applicants respectfully traverse this rejection.

Jahoda et al. teach that the transplantation of low passage dermal papilla cells in rat ear wounds resulted in the production of hair growth in comparison to a control of transplanted skin fibroblasts and later passage dermal papilla cells. In Jahoda et al., the cells are scraped from the base of a dish with a rubber policeman and removed in clumps which are then pushed into the wound gap. (page 585-586). Thus, Jahoda et al. does not teach the creation of a cultured tissue construct comprising cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured cells and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic support members during the culturing conditions.

As discussed above, Parenteau et al. does teach a chemically defined cell culture medium, but does not teach or suggest the instantly claimed invention. The Office Action points to Example 5 of Parenteau et al. as demonstrating that Parenteau et al. teaches the culturing of human keratinocytes on plates not coated with collagen. Example 5 of Parenteau et al. relates to a study to determine actual substrate dependence and the effect of substrate over culture lifespan. The results of this study indicate that cells plated on dishes with collagen generally performed better than those plated on dishes without collagen. The study found that: (1) cells established from frozen primary cultures without collagen showed a slight decrease in plating efficiency over those plated on collagen; (2) as the cells without collagen were further passaged onto dishes with and without collagen, the plating efficiency was consistently better on those plated onto dishes with collagen; (3) cells initially maintained with collagen and subsequently passed onto dishes with or without collagen showed variable plating efficiencies without clear advantage or disadvantage to the use of collagen; and (4) cells grown and passed onto collagen took longer to show a drop in plating efficiency than those grown without collagen.

An additional experiment discussed in Example 5 relates to a strain of epidermal cells in high serum with 3T3 feeder cells. As discussed earlier in the specification of Parenteau et al. at column 2, lines 38-58, this method uses a 3T3 feeder cell layer (from a transformed mouse cell

line) to allow clonal growth and multiple passage and therefore does not teach the claimed invention.

Therefore, Example 5 of Parenteau et al. teaches that cells plated on dishes with collagen performed better than those plated on dishes without collagen. Furthermore, Parenteau et al. states that "[v]arious substrates can be used in the practice of the present invention . . . . In the claimed systems, growth may be enhanced under certain conditions using either fibronectin or collagen coated substrate . . . . The presence of a substrate, e.g., a matrix component appears to allow the cells to establish colonies when conditions are less than optimal or more stringent . . . ." (col. 18, line 63 to col. 19, line 16). Thus, one would not be motivated to combine the Parenteau et al. reference with Jahoda et al. in order to culture cells without collagen because Parenteau et al. would suggest the use of collagen, not its absence, as being advantageous. Parenteau et al. do not teach that the cells cultured in Example 5 produce their own matrix. Parenteau et al. do not show a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells or an extracellular matrix produced by the cultured fibroblast cells.

Parenteau et al. generally teaches the production of a culture of epidermal cells. In Parenteau et al. a living skin equivalent was fabricated by plating keratinocytes onto a dermal equivalent made according to established procedures (col. 26, lines 1-4), thus using exogenous matrix components. The tissue cultures in the instant invention produce their own extracellular matrix and form a tissue construct. The ability of fibroblast cells to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members was unexpected. The cells cultured in Example 5 of Parenteau et al. would produce merely a multitude of epidermal cells, not a tissue construct. Therefore, Parenteau et al. do not teach a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells wherein the extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members.

Thus, it would not have been obvious to one of ordinary skill in the art at the time of the invention to create a cultured tissue construct comprising fibroblast cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured

cells and wherein said extracellular matrix is produced in the absence of exogenous matrix components or such a construct wherein said cultured cells are dermal papilla cells with or without a top layer of epithelial cells.

Therefore, Applicants submit that the cited references do not, either alone or in combination, render the claimed invention obvious. For this reason, the Examiner is requested to reconsider and to withdraw this rejection.

IV. Conclusion.

Applicants respectfully submit that all the bases for rejection of the pending claims are now moot. The Examiner is requested to reconsider the rejections and to withdraw them and to pass this case to issuance.

Respectfully submitted,

Collen Sypherd #39,850 for Hollie Baker

Hollie L. Baker
Registration No. 31,321

Date: October 3, 2001

Hale and Dorr LLP
60 State Street
Boston, MA 02109
617 526-6110 (Telephone)
617 526-5000 (Fax)

Attachments: Copy of Pending Claims amended with this Response
Marked-Up Copy of Amended Claims

Marked-Up Version of Amended Claims
U.S.S.N. 09/523,809
Filed March 13, 2000
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A cultured tissue construct comprising fibroblasts cells grown under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

2. The cultured tissue construct of claim 1, wherein fibroblasts are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, urethra, umbilical cord, corneal stroma, oral mucosa, and intestine.

3. The cultured tissue construct of claim 1, wherein said cultured cells are dermal fibroblasts.

4. The cultured tissue construct of claim 1, wherein said cultured cells are from dermal papilla of hair follicles.

5. The cultured tissue construct of claim 1, wherein said layer has cultured cells from dermal papilla of hair follicles are localized on said layer.

6. The cultured tissue construct of claim 1, wherein said cultured cells are cultured in chemically defined media.

7. The cultured tissue construct of claim 1, wherein said cultured cells are derived from human tissue and are cultured in medium containing no non-human components.

8. A cultured tissue construct comprising cultured dermal fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin,

(iii) fibronectin

(iv) tenascin; and,

(v) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

9. A cultured tissue construct having at least two layers, comprising:

(a) a first layer of cultured fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of cells comprising epithelial cells disposed on the first layer.

10. The bilayered cultured tissue construct of claim 9, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

11. The bilayered cultured tissue construct of claim 9, wherein said fibroblast cells contained within said first layer are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, and intestine.

12. The bilayered cultured tissue construct of claim 9, wherein said fibroblast cells contained within said first layer are dermal fibroblasts.

13. The bilayered cultured tissue construct of claim 12, wherein said fibroblast cells contained within said first layer are from dermal papilla of hair follicles.

14. The bilayered cultured tissue construct of claim 9, wherein said first layer has cultured cells from dermal papilla of hair follicles are localized on said first layer.

15. The bilayered cultured tissue construct of claim 9, further comprising a third layer of cells disposed on the second layer of epithelial cells.

16. A cultured skin construct having at least two layers, comprising:

(a) a first layer of cultured dermal fibroblasts cells cultured under conditions to produce a layer of extracellular matrix which is synthesized and assembled by the cultured fibroblast cells, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) fibronectin,

(iv) tenascin; and,

(v) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions; and,

(b) a second layer of keratinocyte cells disposed on the first layer to form an epidermal cell layer, wherein the epidermal cell layer is a multilayered, stratified, differentiated and exhibits a basal layer, suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

17. The construct of any of claims 1, 8, 9, and 16, wherein the cultured fibroblast cells are genetically modified to produce extracellular matrix components.

18. The construct of claim 17, wherein the cultured fibroblast cells are genetically modified to produce a growth factor, hormone, peptide, or protein.

19. (Amended) A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a first cell culture medium;

(b) culturing the fibroblast cells of step (a) in the first cell culture medium to between about 80% to about 100% confluence on the porous membrane;

(c) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components ~~under~~ **by** culturing ~~conditions~~ **the cells** in a second culture medium; and,

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

20. The method of claim 19, wherein either the first culture medium, or the second culture medium, or both the first and second culture mediums are chemically defined.

21. The method of claim 19, wherein the first and second culture mediums contain no non-human components.

22. The method of claim 19, wherein in step (a) the fibroblast cells are seeded at a density between about 1 x 10^5 cells/cm^2 to about 6.6 x 10^5 cells/cm^2.

23. The method of claim 19, wherein the fibroblast cells are derived from tissue selected from the group consisting of neonate male foreskin, umbilical cord, dermis, tendon, lung, urethra, corneal stroma, oral mucosa, and intestine.

24. (Amended) A method for producing a bilayered cultured tissue construct, comprising:

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a first cell culture medium;

(b) culturing the fibroblast cells of step (a) in the first cell culture medium to between about 80% to about 100% confluence on the porous membrane;

(c) stimulating the fibroblast cells of step (a) to synthesize, secrete and organize extracellular matrix components ~~under~~ **by** culturing ~~conditions~~ **the cells** in a second culture medium;

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of between about 30 to about 110 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions;

(e) seeding epithelial cells to the top surface the synthesized extracellular matrix of step (d), and,

(f) stimulating the epithelial cells of step (e) under culturing conditions to form a bilayered tissue construct of an extracellular matrix, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, and a second layer of epithelial cells.

25. The method of claim 24, wherein said fibroblast cells capable of synthesizing an extracellular matrix are derived from tissue selected from the group consisting of neonate male foreskin, dermis, tendon, lung, cartilage, urethra, corneal stroma, oral mucosa, and intestine.

26. The method of claim 24, wherein the epithelial cells are selected from the group consisting of keratinocytes, corneal epithelial cells, epithelial cells from oral mucosa, esophageal epithelial cells, and uroepithelial cells.

27. A method for producing a bilayered cultured skin construct comprising a dermal layer and an epidermal layer disposed thereon in the absence of a structural support scaffold or exogenous matrix components, wherein the method comprises the steps of:

(a) seeding fibroblast cells on porous membrane in a culture vessel in a first chemically defined cell culture medium at a density between about 1 x 10^5 cells/cm^2 to about 6.6 x 10^5 cells/cm^2;

(b) culturing the fibroblast cells of step (a) in the first chemically defined cell culture medium to between about 80% to about 100% confluence;

(c) stimulating the fibroblast cells of step (a) to synthesize, secrete and organize extracellular matrix components by culturing the cells in a second chemically defined culture medium;

(d) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer,

wherein the extracellular matrix comprises:

(i) type I and type III collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) decorin;

(iii) tenascin; and,

(iv) glycosaminoglycans;

wherein said extracellular matrix is produced by the cultured dermal fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions;

(e) seeding keratinocyte cells to the top surface of the synthesized extracellular matrix of step (d), and,

(f) culturing the keratinocyte cells under culturing conditions to form an epidermal layer,

wherein the epidermal cell layer is a multilayered, stratified, differentiated layer of keratinocytes that exhibit a basal layer, a suprabasal layer, a granular layer and a stratum corneum;

and wherein the bilayered cultured skin construct has a basement membrane present at the junction of the first and second layers.

28. A method for transplantation or implantation of a cultured tissue construct into a patient comprising transplanting or implanting a cultured tissue construct of any of claims 1, 8, 9 or 16 into a patient in need of treatment thereof.

29. A method for producing a cultured tissue construct, comprising,

(a) seeding fibroblast cells capable of synthesizing an extracellular matrix on a porous membrane in a culture vessel in a cell culture medium at about 80% to about 100% confluence;

(b) stimulating the fibroblast cells to synthesize, secrete and organize extracellular matrix components under culturing conditions in a second culture medium; and,

(c) continued culturing of the fibroblast cells until the cells form a layer of synthesized extracellular matrix of at least about 30 microns thick, with the cultured fibroblast cells contained within the synthesized extracellular matrix layer, wherein the extracellular matrix comprises:

(i) fibrillar collagen showing a packing organization of fibrils and fibril bundles exhibiting a quarter-staggered 67 nm banding pattern;

(ii) tenascin; and,

(iii) glycosaminoglycans;

and wherein said extracellular matrix is produced by the cultured fibroblast cells in the absence of exogenous matrix components or synthetic members during the culturing conditions.

30. The construct of any of claims 1-18, wherein the construct is cohesive in having physical unitary integrity and tissue-like handling properties.